4. INTRODUCTION

4.1. Investigational Plan

Vical Inc. proposes to investigate the effect of a combination regimen with the direct gene therapy immunotherapeutic, Allovectin-7, and the chemotherapy agent dacarbazine (DTIC) in patients with metastatic or recurrent melanoma. Several studies in recent years have shown an additive effect when chemotherapy agents and immunotherapeutic agents are used in various combinations in this patient population. Response rates as high as 60-70% have been reported with combinations of chemotherapy, recombinant interferon α (rIFNα) and recombinant interleukin-2 (rIL-2) (1,2). Our studies with the gene therapy immunotherapeutic, Allovectin-7, indicate that restoring the MHC class I antigen expression on the tumor surface may trigger a cytotoxic T lymphocyte antitumor response both within the injected tumor and at distant sites, causing tumor regression in certain patients with advanced metastatic disease after either failure or relapse from chemotherapy regimens. It therefore seems appropriate to evaluate the safety and efficacy of Allovectin-7 at an earlier stage of disease in combination with a chemotherapy regimen with the hope of achieving either a higher response rate and/or more durable responses and/or a longer median time to disease progression. Allovectin-7 may also provide a less toxic alternative to systemic rIL-2 and subcutaneous rIFNα therapy.

4.2. Overview

Cutaneous melanoma has one of the most rapidly increasing incidence rates of any cancer in the U.S. Early detection and surgical excision of melanomas can be curative, but once the tumor spreads beyond the skin, it is one of the most deadly forms of cancer. There are currently no effective therapies for advanced disease and l0-year survival rates for these patients are very low (3,4).

Dimethyl triazeno imidazole carboxamide (dacarbazine or DTIC) is the best studied chemotherapeutic agent for the treatment of metastatic melanoma with a response rate in the range of 15-25% (5). The median duration of response is 5.5 months; the median time to treatment failure is 2.6 months. In 580 patients entered into Phase III trials, only 5% complete responses were observed (6). Only 31% of patients who achieved complete response remained disease free at 6 years. Long-term complete responses were noted in only 1-2% of patients treated with DTIC. Many other chemotherapeutic agents have also been evaluated as single agents in patients with metastatic melanoma (7) as shown in Table 1.

Table 1. Response Rate to Single-Agent Chemotherapy in Metastatic Melanoma

AGENT	n	CR+PR (%)	95% CI (%)
Dacarbazine (DTIC)	1936	382(20)	18-22
Carmustine (BCNU)	122	22(18)	11-25
Lomustine (CCNU)	270	35(13)	9-17
Tauromustine (TCNU)	42	7(17)	6-31
Fotemustine	153	37(24)	17-31
Cisplatin (CDDP)	188	43(23)	17-29
Carboplatin	43	7 (16)	5-27
Vincristine	52	6 (12)	3-20
Vinblastine	62	8 (13)	5-21
Vindesine	273	39 (14)	10-18
Taxol	65	12(18)	9-28
Dibromodulcitol	205	28(14)	9-18
Doxorubicin	42	8 (19)	7-31
Piritrexim	31	7(23)	8-37

CR = complete response; PR = partial response; CI = confidence interval

More recently, several multi-drug regimens containing DTIC and/or cisplatin have been evaluated (Table 2) (8).

Table 2. Selected Combination Chemotherapy Regimens

SOURCE	REGIMEN	n	CR+PR	RESPONSE RATE (%)
Costanzi et al.	DTIC+BCNU	61	12	20
Costanzi et al.	DTIC+Me-CCNU	122	18	15
DeWasch et al.	CCNU+VCR+BLM	42	17	40
Seigler et al.	DTIC+CCNU+VCR+BLM	72	29	40
York et al.	DTIC+CCNU+VCR+BLM	46	10	22
Costanzi et al.	DTIC+BCNU+HDU	95	29	31
Fletcher et al.	DDP+DTIC	30	11	37
Restas et al.	DTIC+VDS	46	10	22
Mulder et al.	DDP+VDS	61	13	21
Nathanson et al.	DDP+VLB+BLM	42	18	43
Verschraegen et al.	DDP+DTIC+VDS	105	22	21
Ringborg et al.	DDP+DTIC+VDS	40	15	38
Legha et al.	DDP+DTIC+VLB	50	20	40
Del Prete et al.	DDP+DTIC+BCNU+TAM	20	11	55

Abbreviations: BLM, bleomycin; CR, complete response; DDP, cisplatin; HDU, hydroxyurea; Me-CCNU, methyl-CCNU; PR, partial response; TAM, tamoxifen; VCR, vincristine; VDS, vindesine; VLB, vinblastine.

A three-drug combination developed at M.D. Anderson that has shown a promising response rate is CDDP/VLB/DTIC (CVD). This regimen has produced responses in 40% of patients with most responses being partial with a median response duration of 4-6 months (9). A four-drug regimen pioneered at Dartmouth is a combination of DTIC, CDDP, BCNU and Tamoxifen and has been reported to have a 50% response rate (10). Unfortunately, all of the multiple drug regimens result in increased toxicity. There is no consensus on second line treatment, and no treatment after front-line therapy for Stage III and IV disease has been shown to be effective. Overall, the role of combination chemotherapy remains controversial and its impact on long-term survival has been disappointing. Other approaches being explored include the use of cytokines or other experimental approaches.

The role of the immune system in malignant melanoma has been an area of intense interest. Melanoma antigens have been well studied in the past, as has evidence of a host immune response (5,6). Agents that can stimulate non-HLA-restricted cellular cytotoxicity, such as interferons and interleukin-2, have produced sustained regressions in some patients (11). The toxic side effects of these agents, however, are considerable, with several deaths associated with the use of high dose regimens.

It is now possible to trigger an immune response through gene transfer. Numerous models have been developed and have led to several clinical trials exploring the possibility of manipulating either tumor cells or host lymphocytes transfected with a variety of cytokine genes (12). Other specific gene therapy strategies have sought to directly influence the interaction between immunocyte and antigen presenting cell by enhancement of HLA-restricted immunity.

5. BACKGROUND AND RATIONALE

5.1 Chemotherapy / immunotherapy combination studies

Within the last few years, data has been accumulating to support the use of chemotherapy and biotherapy administered together or sequentially. The clinical rationale behind this approach is derived from studies with rIL-2 and rIFNα where a lack of cross resistance in patients treated with cytotoxic agents was observed, i.e. the response rates observed with the immunotherapeutic were not dependent on whether a patient had previously received chemotherapy. Also, various studies have now shown that a biotherapy/chemotherapy combination regimen results in a synergistic or additive effect (1,2). For example, the use of rIFNα and rIL-2 in some studies has resulted in an additive antitumor effect with response rates boosted from 15-40% to as high as 60-70%. Significantly, the number of complete responses also increased from 5 - 10% for chemotherapy alone to 13-24% for the chemo-biotherapy combination. Duration of response has also increased. S. Legha and colleagues at M.D. Anderson Cancer Center recently completed extensive studies where the regimens were altered in

various ways to determine which combination (chemo first, biotherapy first, or an alternating regimen) yielded the best response rates. The chemotherapy drugs used included cisplatin, dacarbazine and vinblastine (CVD). The study results indicated that a CVD / rIFNα + rIL-2 regimen in 30 patients yielded a 66% response rate compared to a 40% response rate in the 30 patients who received the rIFNα and rIL-2 first and then the CVD. Blair et al used DTIC and cisplatin followed by rIL-2 and found a 41% response rate; Khayat et al gave cisplatin followed by 2 courses of rIFNα and rIL-2 with a 41% response rate; Richards et al used cisplatin, carmustine, DTIC, and tamoxifen using a regimen very similar to Legha's and had a 57% response rate. Additional studies are in progress which may provide more definitive answers, but not for 3-5 years (2).

It is important to note that the toxicities associated with these chemo-biotherapy regimens are pronounced. Most of the studies conducted so far have been in highly compromised patients with Karnofsky performance scores as low as 40% and even though the risk of fatal complications may be controllable during therapy, the fact remains that undergoing a regimen with chemotherapy, rIFNα and rIL-2 together can result in more debilitation and prolonged hospital stays in patients who already have a very limited potential life span (13).

The use of the gene therapy immunotherapeutic, Allovectin-7, may provide a less toxic, but potentially equally effective alternative to the use of rIFNα and rIL-2 in a chemo-biotherapy combination therapy. Even if the rate of response were not to improve using Allovectin-7, the use of this immunotherapeutic in combination with DTIC may however, either extend the time to disease progression, or produce more durable responses resulting in tangible clinical benefit to patients.

5.2 Experience With Allovectin-7

Allovectin-7 is a direct gene therapy product developed by Vical Inc. which contains the gene for the highly immunogenic MHC class I transplantation antigen HLA-B7. It is administered by direct intratumoral injection. The product contains plasmid DNA, VCL-1005, which encodes the HLA-B7 heavy chain and β2 microglobulin proteins. The β2 microglobulin allows synthesis and expression of the complete MHC complex on the cell surface (14). The plasmid is complexed with the cationic lipid mixture, DMRIE / DOPE (2,3-bis(tetradecyloxy)propyl,2-hydroxyethyl,dimethylammoniumbromide / dioleoyl phosphatidylethanolamine), which facilitates transfection of the plasmid DNA. Qualitative or quantitative changes in MHC class I antigens on the tumor cell regulate the sensitivity of the tumor to immunological rejection by autologous T lymphocytes (1). In experimental tumor models (15-17), variation in the expression of MHC class I antigens has been shown to exert a decisive influence on local tumor growth and metastases. Total or selective loss of MHC class I antigens have been reported in 10%-30% of biopsies of human melanoma lesions (18). In some cases, a correlation was found between HLA loss and poor prognosis (19).